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Nitrogen partition in repeated fasting

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NITROGEN PARTITION IN REPEATED FASTING

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B. S. University of Illinois, 1906

M. A. University of Illinois, 1907

THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Paul Edward Howe

ENTITLED Nitrogen Partition in Repeated Fasting

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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on

Final Examination



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NITROGEN PARTITION IN REPEATED FASTING.

Historical.

The literature upon fasting is large and may be divided into two classes, that presenting data upon: A, Simple fasts of (1) short duration, and dealing with changes in body weight, nitrogen partition, the blood, temperature, secretions, and the effect of drugs, or only as a preparation for the study of normal or pathological metabolism; (2) more or less profound fasts, which include in addition to those changes mentioned above, also, losses in the weight and differences in composition of the organs as compared to the normal organs: and, B, Repeated fasts in which (1) the object was to study the effect of repeated fasting; or (2) fasts in which the main object was not the study of repeated fasting, but in which the subject was used repeatedly in various investigations, no regard being given to the original condition, such as body weight, nitrogen equilibrium, etc.

Experiments on Repeated Fasting: Investigations upon fasting have been limited mainly to the lower animals, while those upon repeated fasting are confined entirely to them unless the fasts of Succi¹ are to be considered as repeated fasts, which is hardly proper, since each fast was conducted under different circumstances as regard original condition, body weight, time of year, climate, etc.

Repeated fasts have been conducted upon dogs by Albitsky³. His investigations were made upon four dogs, two fasting absolutely i. e. without water, to a loss in body weight of 40.4 per cent. and 37.6 per cent, in twenty-nine days, and fifteen days respectively, and the second time with water ad libitum, with

a loss of 53.2 per cent. and 45.8 per cent, in fifty-one, and thirty days, respectively. The other two dogs fasted first with water, to losses of 41.5 per cent. and 38.7 per cent. in thirty-four days and twenty-five days, respectively, and the second fast to a loss of 43.7 per cent. and 49.0 per cent. body weight, in thirty-two days, and thirty-eight days, respectively. Positive conclusions cannot be drawn from Albitsky's results. No study was made of the effect of a properly controlled repeated fast either complete or with water. Hence he had no basis upon which to compare the effect of water upon the length of a fast. A study of the results show in general, that water seems to lessen the breakdown of the body tissues, for in three out of the four cases the dogs fasted a greater length of time for the same loss in body weight when they received water than when no water was given.

Tuvim⁴ subjected two dogs to three repeated fasts of three days each, the first fast being absolute, and the two following with a large water ingestion. A third dog was fasted but twice, the first fast being absolute and the second with water. His experiments were to study the effect of water upon fasting, and only the respiration data were observed. His results did not show that water had any pronounced effect upon the gas interchange; and no data are presented which would show the effect of repeated fasting upon the nitrogen partition.

Schulz working with Mangold⁹ Stübel¹⁰ and Hempel¹¹ reports results upon repeated fasting in dogs. One dog weighing 16.1 kilograms was fasted for thirty-two days, when the premortal rise seemed to have set in, and then the animal was fed an insufficient nitrogenous diet for nine days, during which time it stored 23 grams of nitrogen. This nitrogenous feeding was followed by a seven day carbohydrate feeding period with a loss of 12.93 grams of nitrogen, leaving a storage of 10.07 grams of nitrogen for the sixteen days, during which the animal was fed. This feeding period was followed by another fast of fourteen days with a further loss of 27.61 grams of nitrogen over that gained and lost during

the feeding.

This same dog was subjected to a similar experiment one month later. At the beginning of this experiment the animal weighed 19.6 kilograms and probably had greater fat stores than in the first experiment. The dog was fasted for twenty-seven days, at the end of which time he was so reduced in strength that they feared he would die. Schulz described the condition as a collapse. Food was given, 400 cc. of milk on the first day and 300 grams of meat per day for four days, restoring the dog to a satisfactory condition, and after which the dog was fasted further for sixty-one days, at the end of which time, the animal was very weak, although no premortal rise had occurred. During this second series of fasts the dog had lost 208.4 grams of nitrogen without regard to the nitrogen balance of the feeding period, while in the first series of fasts the dog lost 183.2 grams of nitrogen. The daily average nitrogen excretion during the second part of the first series and the first part of the second series was less than that in the first fast, while the average nitrogen excretion in the final fast of sixty-one days was even lower than the nitrogen excretion of the feeding period when sugar was ingested.

Stübel¹⁰, working on another dog obtained a similar lowered nitrogen excretion after a short feeding period in which the dog received an insufficient food supply.

Manassein⁵ reports experiments upon the repeated fasts of three rabbits in absolute fasting, and Albitsky³ upon nine rabbits fasting either absolutely or alternately with water. In Albitsky's experiments the object was the study of the effect of water upon fasting and the following conditions were in force in his work. The first three rabbits were fasted absolutely while the remaining six rabbits were fasted alternately with and without water. The fasts were continued to a loss of 40 per cent. of the body weight and it was found that with each succeeding fast there was a tendency toward a decrease in the daily loss in

body weight. Albitsky also observed that the recovery was slower with each succeeding fast and that as a result of fasting, the animals' "oxidative" properties were increased so that from one to one and one-half times as much food and twice as much water were needed to keep the animal in a state of constant body weight. Pashutin² concludes from the work of these men that with the ingestion of water there is a slower loss in body weight than would occur in absolute fasting. The conditions of experimentation were not controlled accurately in these experiments and only general deductions may be drawn from the results.

Kagan⁶ conducted experiments in repeated absolute fasting upon pigeons and showed in birds results similar to those obtained upon dogs and rabbits, i. e. a slower loss in body weight upon repeated fasting. This decrease in the loss of body weight does not continue with each succeeding fast but remains fairly constant. Albitsky³ reports results upon pigeons which confirm his findings upon rabbits. He also gives data showing the influence of water upon fasting.

Ugrumoff⁷ in two experiments upon pups showed that water exerted a sparing influence upon the organs over the loss found by fasting when a salt solution was given. Sadoven⁸ working along the same lines subjected rabbits to repeated fasts under varying conditions such as absolute, with water, or with salt solution. He showed that salt solution exerted a sparing influence upon the loss of body weight as compared with complete fasting, while water exerted a still greater influence.

Bischoff and Voit¹³ report a number of fasts, twenty-eight, upon one dog, under widely varying conditions, no single fast being more than eight days long and some were for only one to two days.

Seeland¹⁴ conducted experiments on short repeated fasts with pigeons and roosters and showed that those birds which were subjected to repeated fasts of one to three days duration gained weight more rapidly, tissues were more solid, the birds hardier and stronger than the check birds which were fed every day.

Richet¹⁵ fed and fasted rabbits alternately in periods of six days; under such treatment they died in about a month and a half. Dogs subjected to a similar treatment lived about six months.

The work upon repeated fasting which has been cited cannot be interpreted in any way to show the effect of repeated fasting with water. The only cases in which the animals were fasted repeatedly and absolutely were those of Albitsky's rabbits which were fasted to a loss of 40%, of the body weight. The most of the data presented deal largely with the changes in body weight in some cases the respiration data, urine volume, P_2O_5 and urea excretion were determined but no accurate data are presented upon nitrogen partition.

Short repeated fasts upon men are reported by Bucker¹⁶, Smith¹⁷, Ranke¹⁸, Pettenkofer and Voit¹⁹, Oppenheim²⁰, Sadoven²¹, and Richet²².

Accurate studies of the nitrogen partition in fasting dogs have been made but recently. Previous to the advent of Folin's³² method for creatine and creatinine no accurate investigations were made showing the nitrogen partition further than urea and ammonia, and in one or two cases the excretion of some of the pathological constituents of the urine.

Osterberg and Wolf³³ report the nitrogen partition in an experiment in which a dog was fed a low protein diet for a number of days and was then given a large amount of casein, after which the animal was fasted. Their findings cannot be considered normal nitrogen partition data for a fasting dog, inasmuch as the ingestion of a large amount of protein following a low protein diet would influence the nitrogen partition of the succeeding fasting days. Underhill and Kleiner³⁴ report findings upon the nitrogen partition in the urine of a fasting dog. Their experiment extended over a period of fourteen days, water being taken in varying amounts. Their results upon the nitrogen distribution during fasting as compared with a well fed animal show (1) "A slightly increased percentage of ammonia nitrogen and a correspondingly diminished urea percentage." (2) The

total creatinine-nitrogen does not bear a strict relation to the total nitrogen
-----"nor is the quantity excreted constant." "The relatively large output
of creatine is also of special interest although its significance at this time
is obscure." They report the presence of allantoin in the urine and also allanto-
toin crystals in the urine after standing, and hold that the presence of this
substance in "the urine of starving dogs shows without question that allantoin
may have an endogenous origin." With regard to the purine and allantoin excre-
tion they say that; "In inanition no fixed relationship appears to exist between
the excretion of purine and allantoin.

Schöndorf,³⁵ working with a fasting dog shows that the percentage of urea
nitrogen may fall as low as 75 per cent. of the total nitrogen.

Leffman³⁶ reports results which show that the creatine and creatinine ex-
cretions are fairly constant for animals of equal body weight, and that creatine
or creatinine ingested with food, by a well nourished dog, are seemingly excre-
ted as such. Weber³⁷ shows that in fasting dogs as a result of muscular activ-
ity creatinine is excreted, while with uniformly nourished animals creatinine
diminishes. Dorner³⁸ showed that fasting had no influence upon the excretion
of creatinine in rabbits but that creatine appeared in increasing amounts.
Böhtlingk³⁹ gives results upon the nitrogen distribution in fasting cats and
rabbits.

The more recent work upon fasting which deals with the occurrence of crea-
tine and creatinine has been presented by van Hoogenhuyze and Verploegh⁴⁰,
Benedict⁴¹, Benedict and Diefendorf⁴², and Cathcart⁴³ who report results which
are in general quite similar, the creatine remaining fairly constant, with a
slight decrease as the fast progresses. The appearance of creatine in increas-
ing amounts is reported by all except van Hoogenhuyze and Verploegh, who did
not determine creatine. Benedict and Diefendorf report a decrease in creatine
after the rise.

Results upon single prolonged fasts in the case of dogs have been reported by following investigators. Howe, Mattill and Hawk²³, Falok²⁴, Kumagawa and Miura²⁵, Pfluger²⁶, Daddi²⁷, Grandis²⁸, Krawkaw²⁹, L'abbé and Vitry³⁰ and Luciani and Bufalini³¹. The longest normal single fast has been reported by Howe, Mattill, and Hawk²³. In this fast their dog "Oscar" fasted 117 days during which time he received a constant water ingestion. The next longest normal fast upon which data are presented was reported by Falok²⁴. This fast was continued for 60 days. Kumagawa and Miura²⁵ report a fast of 98 days during which time the dog was subjected to repeated doses of phloridzin.. This fast cannot be compared with true fasts because the phloridzin introduces unknown factors.

Description.

Purpose and Plan:--The purpose of this investigation was to study the effect of repeated fasting upon the nitrogen partition in the urine of a dog. Changes in body weight were also observed. As a basis of comparison the nitrogen partition of the urine was determined during a normal feeding period for a number of days preceding the fast.

The plan of the experiment was as follows: The dog was brought into nitrogen equilibrium and then permitted to fast, the fast continuing until the inception of the premortal rise. At this time the animal was fed, the feeding continuing until the dog had regained its original body weight and was again in nitrogen equilibrium. Following this came the second fast.

Subject.--The animal used in this experiment was a small fox-terrier bitch, from one to two years old and weighing 3.41 kilograms. She was in good physical condition, well muscled, and had a medium layer of fat under the skin. Life in the experimental cage did not seem to annoy her in any way.

Articles of Diet.--Meat.--The meat fed was beef, which had been prepared according to the method proposed by Gies⁴⁴ and modified by Hawk⁴⁵. This method of preparation and preservation consisted in freeing the meat from as much visible fat and connective tissue as possible. The trimmed meat was ground three times in a sausage mill, using a smaller plate each time, the meat was passed through the mill and mixed thoroughly between each grinding. This insured a uniform sample. After the juices had been pressed out of the meat it was sampled and then wrapped in paraffin paper in cakes of about 400 to 600 grs., placed in a museum jar, frozen and kept in cold storage. This method of preservation was very satisfactory, the meat remained sweet, and after a period of six months the composition as regards the nitrogen content was practically the same. The analysis of the meat at the time of preparation showed 3.62 per cent. nitrogen and after a period of six months showed 3.69 per cent. nitrogen, or on a moisture

free basis when prepared 4.88 per cent.; and after six months 4.86 per cent. of nitrogen.

Cracker Dust.-Cracker dust was purchased already ground from the manufacturer. This was thoroughly mixed, sampled, analyzed, and closed in air-tight jars.

Lard.-Pure leaf lard was used.

Bone ash.-A good quality of finely ground bone ash was used.

Water.-Ordinary distilled water was used, such as is supplied to the chemical laboratory.

Method of Collection and Preservation of Excreta, etc.-The animal was kept in an experimental cage such as described by Gies⁴⁶ and modified by Hawk⁴⁷. This cage was provided with a wire netting bottom of about 3/8 inch mesh. Below this wire bottom, upon which the animal stands, there is arranged a sliding pan, having sides which slope at an angle of about thirty degrees. An opening was provided at the lowest point through which the urine might pass into a graduated receptacle hung from the bottom. With such a cage the urine and feces could be collected separately, the urine flowing into the receiver as soon as passed, where it was preserved by means of thymol. The feces which were, in the feeding periods, hardened by means of the ingested bone ash, remained on the wire netting and were picked out with a pair of forceps. The hair and scurf fell through the netting onto the pan and were brushed off each day. At the end of each period the whole cage was carefully washed. The cage washings consisted of scurf and small quantities of soft feces which had fallen through the netting.

The urine was collected in 24-hour periods, kept in tincture mouth glass bottles, with ground glass stoppers. The dog was not catheterized for it was found that when the animal was allowed to urinate at will that the bladder was satisfactorily emptied each day or, if a portion of the dog's urine was re-

tained this was a fairly constant quantity. An examination of Table I, page shows that the urine volumes were as a rule very close to 250 cc. per day, corresponding to a daily water ingestion of 250 cc. The other factors, such as the formation of water in metabolism, and loss through respiration would seem to compensate each other. Thymol and refrigeration⁴⁷ were used as means of preservation.

The feces were collected when passed, dried, pulverized, the hair separated, after which the feces were analyzed. Analyses were made in periods. The hair, scurf, and cage washings were also analyzed in periods and the nitrogen distributed evenly according to the number of days in the period.

The dog was weighed on a platform balance which was accurate to 0.01 kilogram.

Analyses and Methods.--Total nitrogen, urea, creatinine, creatine, allantoin, and purine determinations were made upon the urine, and total nitrogen determinations upon the feces, hair and cage washings. All urine analyses were made in duplicate and upon the 24-hour sample, except for a part of the intermediate feeding period, when analyses were made on two-day composite samples, and, in the case of allantoin and purine, which were determined in composite samples of from two to ten days.

The total nitrogen was determined by the Kjeldahl method, copper sulphate⁴⁸ being used in the preliminary oxidation.

The urea was determined by the Benedict-Gephart⁴⁹ modification of the Folin⁵⁰ method. While this method has recently⁵¹ been shown to be slightly inaccurate, due to the partial decomposition of uric acid, creatine, creatinine, allantoin, and hippuric acid, still these substances occur in such small amounts in the urine of the dog, that the urea results may be accepted as a fairly accurate indication of the amount of urea present in the urine. Ammonia was determined by the method described by Folin⁵². Creatinine was also determined by the

Folin³² procedure. The creatine was hydrolyzed in the autoclave as suggested by Benedict and Meyers¹¹. Allantoin and purine were determined upon the same composite sample by the method suggested by Podushka⁵⁴, and modified by Underhill and Kleiner³⁴.

Blank determinations were made in all cases where possible impurities in the reagents might cause an error.

Experimental.

Preliminary Feeding Period.

In an investigation of this character in which the nitrogen distribution is the subject under consideration it is important that there shall be a preliminary period. During this time the subject will live under the same conditions as those which will exist during the experiment proper, and will receive a constant diet. In our case in addition to obtaining uniform conditions of living and diet, this period served to put the animal in a condition of nitrogen equilibrium, and also to furnish data for the nitrogen partition of the animal under these normal conditions, and thus serve as a basis of comparison for the fasting and for the intermediate feeding periods.

The preliminary feeding period in this experiment extended over fifteen days. At the end of this time, during which the animal had received a uniform diet and constant water ingestion, a uniform body weight had been secured and the dog was in nitrogen equilibrium. At this point the nitrogen balance showed an average of -0.271 gram of nitrogen per day for the eight days immediately preceeding the fast. The nitrogen partition in the urine was determined for this period. The diet consisted of 70 grams of meat, 15 grams of cracker dust, 8 grams of lard, 3 grams of bone ash, and 250 cc. of water. See Table VI, page

These ingredients were thoroughly mixed together and fed in the form of a

broth. This diet contained 2.732 grams of nitrogen, or about 0.8 gram per kilo-gram body weight.

The data of the preliminary period are found in Tables I, and IV, pages

From a consideration of the average data for this period it will be seen that with an ingestion of 2.732 grams of nitrogen per day the dog excreted 2.768 grams of nitrogen in the urine, and suffered a further loss of 0.235 gram of nitrogen in the form of feces, hair and cage washings. This gives a minus balance of 0.271 gram of nitrogen per day. This is an approximate nitrogen equilibrium. (See Table IV, page 63).

First Fast.

At the end of the preliminary period the dog was in a fair nitrogen equilibrium, and had maintained a constant body weight for three days. The last food was given at 2 P. M. February fourth, and the fast was considered as beginning at 2 P. M. February fifth. The urine collected at 2 P. M. on February sixth being considered as representing the urine of the first day of fast. On the first day the water (250 cc.) was placed in the food pan, but only 50 cc. were taken. As a result the urine volume for the first day was only 35 cc. and this was combined with the urine of the following day. When the dog refused water on the second day of fast, the fluid was given by means of a tube and this was continued throughout the fasts. On the seventh day the first feces were passed, which were very small in amount and of a pasty consistency. On the eleventh day the second fasting feces were passed; these feces were removed from the cage immediately after defecation and were greenish brown in color, becoming black upon exposure to the air. It seems probable that the fresh fasting feces of the dog are of a greenish brown cast and only assume the black color ordinarily ascribed to them⁵⁶ upon coming in contact with the air. More fasting feces were passed on the thirteenth day of fast. The dog continued

weaker and could hardly stand. Feces were again passed on the fourteenth day, but in small amount.

The lower nitrogen percentages for the feces of the feeding periods above those for the fasting periods (see Table VI, page 75) is probably due primarily to the fact that bone ash was used in the diet of the feeding period thus increasing the bulk of the fecal output although having no influence upon its nitrogen content. At the same time it is no doubt true that under some conditions at least, the percentage of nitrogen in fasting feces is higher than that of the feeding feces from the same animal.[#]

The following entry was made in the diary at the close of the fifteenth day of fast:- February 20th. Soft feces in A. M. Dog could not stand and was in an extremely weak condition. Pulse rose from 54, yesterday, to 140 today, and the temperature sank from 37° C to 36° C. Weight fell from 2.05 Kg. to 1.85 Kg. She has lost over 40 per cent. of her body weight in two weeks. A very striking loss. Dog was so weak we feared to go further, therefore, we fed her (4 P. M.) 15 grams cracker dust, 2 grams bone ash, and 3 grams lard. She took one-half of it. Gave her rest of it in evening, covered her up with a blanket to keep her warm. Her nose is cold. She wagged her tail when addressed, could not stand however (6:10 P. M.). After dinner the dog rose as we entered and walked to the front of the cage, wagging her tail. She was a new dog, and yet she had partaken of but about 8 grams of cracker dust, 3 grams of lard, and 2 grams of bone ash. We fed her the remainder of the amount of food, previously mentioned, and then fixed her blanket for her in the corner of the cage and she lay down contentedly. Later in the evening the dog seemed much stronger".

Discussion of the Nitrogen Distribution for the First Fast.-Total Nitrogen-
From an examination of Table I, page 46, it is seen that in the first two days

[#]Unpublished results from this laboratory.

the nitrogen excretion fell from an average of 2.768 grams during the preliminary period, to an average of 1.312 gram. This low nitrogen excretion is due, as has been proven by Benedict⁴¹ in fasts upon men, to the sparing action of glycogen present in the body. After the second day the nitrogen excretion in the urine rose rapidly from day to day, taking a slight drop during the twelfth to fourteenth days, and followed by a pronounced premortal rise, the nitrogen output increasing from 2.472 grams on the fourteenth day, to 3.944 grams on the fifteenth day.

The rapidly increasing output of nitrogen mentioned above as occurring during the first twelve days of the fast is somewhat unusual. It might be inferred from such data, according to the commonly accepted theories with regard to the nitrogen excretion in the urine of a fasting dog, that the animal was thin and muscular, without any fat stores of any amount. Or, on the other hand it might be argued, that the dog was organically unsound. We believe both premises to be unfounded and have discussed the matter more at length on page

Urea.-The excretion of nitrogen as uræa followed the total nitrogen excretion, increasing absolutely, but not relatively. The percentage urea nitrogen as compared with the total nitrogen was practically constant throughout the fast the average being 89.12 per cent., and the range being 86.36 per cent. on the fifth day to 91.12 per cent. on the sixth day. This average excretion is slightly higher than that found in the normal feeding period (86.79 per cent.).

This constant relation between urea and total nitrogen is contrary to the statements in the literature,^{33,34,35} to the effect that the percentage nitrogen as urea nitrogen decreases as the fast progresses. This fact holds for men^{23,41,43} but does not seem to hold for the dog, as is shown in the experiments reported from our laboratory by Howe, Mattill and Hawk. In their results the variations did not tend in any marked direction away from a constant relative value.

The fact that the method of determining the urea-nitrogen is not absolutely accurate might appear to invalidate our deductions. However, if the method determined all of the nitrogen appearing as creatine, creatinine, etc., the percentage of nitrogen as urea would not be reduced more than 3 to 4 per cent. and this would be a fairly constant quantity throughout the experiment. Therefore this error does not detract from the accuracy of our conclusions.

Ammonia.-The ammonia-nitrogen fell from the average excretion per day of 0.143 gram of nitrogen during the feeding period to an average of 0.103 gram per day during the fast. After the first four days the ammonia excretion remained almost constant rising slightly, at the end, with the premortal rise. The relative excretion increased, then fell, and finally increased again to the end. The range in the percentage of urea-nitrogen as compared to total nitrogen was from 3.64 per cent. on the ninth day, to 6.24 per cent. on the third day, the average per cent. of total nitrogen appearing as ammonia-nitrogen being 4.59 per cent.

Creatinine.-The creatinine decreased in absolute amount very gradually with but slight variations from day to day. The average daily excretion for the fast (0.025 gram of nitrogen) was below that of the feeding period (0.041 gram of nitrogen.) This pronounced drop took place during the first part of the fast and from then on the absolute amount of creatinine excreted per day fell gradually, following in general the decreasing body weight. While the absolute amount of creatinine-nitrogen fell very gradually, the percentage output decreased much more rapidly. The percentage of total nitrogen appearing as creatinine was 2.28 per cent. for the first two days, and then fell very regularly as the fast progressed, to 0.46 per cent. on the last day. The grand average was 1.14 per cent. as against an average of 1.49 per cent. for the normal feeding period.

Creatine.-The creatine-nitrogen fell from the feeding level(daily average of 0.056 gram of nitrogen) to an average value of 0.018 gram for the first two days, and then remained approximately equal to the amount of creatinine nitrogen excreted, being in general somewhat below this value. On the ninth day the amount of creatine-nitrogen excreted rose, very suddenly, from 0.037 gram, to 0.054 gram, and continued on this higher plane, with a tendency toward an increasing excretion, to the end of the fast. The average excretion for these two portions of the fast were 0.025 gram of nitrogen for the first eight days, and 0.068 gram of nitrogen for the last seven days, with a grand average of 0.046 gram of nitrogen.

The percentage of total nitrogen as creatine-nitrogen increased from 1.40 per cent. on the first day to 3.10 per cent. on the twelfth day, due to the greatly increased nitrogen excretion. The average percentage of total nitrogen was 1.14 per cent. against 1.49 per cent. for the feeding period.

Allantoin and Purine.-The allantoin- and purine-nitrogen varied from day to day. The average purine-nitrogen excretion was below that of the feeding period, being 0.005 gram per day, for eleven days of the fast, against 0.012 gram of nitrogen during the feeding period. The same average amount of allantoin-nitrogen as of purine-nitrogen was excreted, i. e., 0.005 gram while there was 0.008 gram of allantoin-nitrogen excreted per day, during the feeding period.

The relative amount of nitrogen excreted as purine- and allantoin-nitrogen were 0.22 per cent. of purine-, and 0.26 per cent. of allantoin-nitrogen, while the feeding period gave 0.45 per cent. purine, and 0.31 per cent. allantoin-nitrogen.

Undertermined Nitrogen.-The undertermined nitrogen fell as a result of the fast, being 0.067 gram for an average during the fast, against 0.109 gram for the feeding period.

Intermediate Feeding Period.

General Considerations.-The object of this feeding period was to restore the animal to its original condition as regards body weight and nitrogen equilibrium. The data upon the income and outgo of nitrogen are found in Tables I and IV, pages 46 and 63. During the portion of the feeding period immediately following the end of the first fast the diet was varied. The same constituents were fed, but the animal did not receive the full diet given in the preliminary period. The diet for the first three days did not contain any meat, and there was a minus balance of 3,000 grams for this period. A positive balance of 1.218 gram occurred on the fourth day, when meat was fed for the first time. During this preliminary period of four days on an extremely low diet, the animal gained 21.08 per cent. in body weight. The original diet was then fed, and continued for fifteen days, during which period the body weight increased but 7.57 per cent. In fact, during the last three days of this period there was practically an increase in weight.

It appeared that to restore the original body weight, on the normal diet, would require a very long time¹, hence the diet was increased 50 per cent. thus causing the dog to ingest 4.104 grams of nitrogen per day. Upon this diet the dog gained 20 per cent. of its weight in eight days. The diet was again increased 50 per cent. at the end of the eight-day period of increased feeding. The animal was now receiving twice as much nitrogen per day (5.464 grams of nitrogen) as was required to maintain her constant body weight at the beginning of the experiment. Throughout these periods of increased diet (50 per cent. and 100 per cent.) the animal showed a plus nitrogen balance. After seventeen

1. That it was possible to restore the normal body weight of fasting rabbits which were fed the original diet following the breaking of the fast, has been shown by Kagan⁶. However, in these tests the animals were not brought into a condition of nitrogen equilibrium before being fasted, and the original diet may therefore have been an excessive one, thus enabling Kagan to restore the normal weight of his rabbits with greater facility than otherwise.

days of the 100 per cent. increase the dog had more than regained the weight lost during fasting, the weight being 3.59 kilograms, against a weight of 3.41 kilograms at the beginning of the fast. The diet was now reduced to the normal (2.732 grams of nitrogen per day). The weight dropped slightly on the first day, and remained constant for the following two days at 3.54 kilograms and at the same time the dog was in nitrogen equilibrium, there being a plus balance of 0.02 gram of nitrogen per day.

In spite of the fact that the dog was giving a minus balance during the first three days of the feeding period she gained 280 grams in weight. This may have been due to the retention of water, for although the dog had been receiving 250 cc. of water daily throughout the fast, upon feeding, the urine volumes for the first two days decreased to 215 and 50 cc. respectively. While the method of collecting the urine (without catheterization) does not give absolute values for the urine volumes, the values indicate that there must have been a marked water retention upon the ingestion of food. This has been previously noted in feeding after absolute fasts in dogs⁹, and also with men²³, who had been receiving water during the fast.

Nitrogen Distribution.—The average relative amount of urea-nitrogen excreted increased, as would be expected, with the increase in the diet, and the amount of nitrogen excreted per day. The percentage nitrogen output as urea dropped to the level of the preliminary period upon the resumption of the normal diet. For further discussion see page 28.

The absolute amount of ammonia-nitrogen increased with the increase in the diet. The relative amount of ammonia decreased, however, and was equal to the normal upon return to the original diet.

The creatinine-nitrogen increased from day to day corresponding to the increase in muscular tissue. The average amount of creatinine excreted upon return to the original condition and body weight at the end of the intermediate

feeding period was practically equal to that excreted under the normal condition in force at the end of the preliminary feeding period, being .040 gram of nitrogen per day, against .041 gram. Relatively, however, the creatinine excretion decreased with the increase in diet and the amount of total nitrogen excreted. On the same diet (normal) the creatinine-nitrogen excretion was 1.49 per cent. of the total nitrogen, during the preliminary feeding period, whereas at the end of the intermediate feeding period, the value was 1.63 per cent. In each case the dog was in nitrogen equilibrium and of practically the same body weight.

The amount of creatine-nitrogen excreted increased with the increase in the quantity of meat fed⁵⁶. In other words the creatine ingested with the food was in part excreted in the urine.

Allantoin- and purine-nitrogen were both excreted in larger amounts than during the fasting period. The average purine nitrogen excretion was less (0.008 gram) than that in the preliminary feeding period (0.012 gram), whereas the allantoin nitrogen was the same, i. e. 0.008 gram.

Second Fast.

At the end of the feeding period, the weight of the animal was constant, within 20 grams, for a period of six days. At the beginning of the second fast the body weight was constant, although a little higher (3.54 kilograms) than at the beginning of the first fast (3.41 kilograms). The nitrogen balance was + 0.02 gram on the day preceeding the fast. The animal appeared to be in a much better physical condition than when brought to nitrogen equilibrium at the beginning of the first fast. The coat was much smoother and the muscles were very firm. The activity and attentiveness were fully as pronounced as at the beginnings of the first fast.

The last food was given at 2 P. M. on April seventh and the urine collect-

ed on April ninth was taken as the first fasting urine. In this fast the water (250 cc. per day) was given entirely by means of a tube. Signs of marked weakness did not appear until about the fourteenth fasting day, and even then the dog was quite active. From then on she became gradually weaker, becoming more quiet and less disposed to move about. She became perceptibly weaker on the twenty-ninth day and at 6:15 A. M. on the thirtieth day of the fast (16 hours after the beginning of that day) the dog was found lying upon her side in a state of coma. Particulars with regard to the post mortem are to be found on page 27.

Total Nitrogen.-The analytical data for the second fast are contained in Table I, page 46. From an excretion of 2.435 grams on the last day of the feeding period, the total nitrogen excretion dropped on the first day of fast to 1.081 gram. This was the smallest twenty-four hour output of nitrogen excreted at any time during the progress of the fast. The nitrogen excretion remained fairly constant for the first twenty-six days. The maximum for this time was 1.471 grams on the sixth day (when no water was given) with an average of 1.231 grams. From the twenty-sixth day on there was an increased nitrogen excretion from day to day, until on the thirtieth day an output of 2.298 grams was registered for a sixteen hour period.

Urea.-The urea-nitrogen excretion followed the total nitrogen. Relative to the total nitrogen the average for the whole fast was 86.51 per cent., the extreme values being 76.56 per cent. and 90.88 per cent. The lower value is probably the result of alkaline fermentation, for the urine was found standing on the pan of the cage, the wire gauze, which served as a filter having become clogged with hair. Aside from three days, the seventeenth, eighteenth, and twenty-third days of the fast on which alkaline fermentation probably occurred, the variation was between 81.95 per cent. and 90.88 per cent with an average of 87.46 per cent., there being no marked tendency toward either a drop or a rise

in the percentage of total nitrogen as urea.

Ammonia.--The ammonia-nitrogen showed a slight tendency to increase, as the fast progressed, both absolutely and relatively. However, after the first six days, the excretion remained fairly constant, there being but slight variations in either direction from the mean (0.083 gram of nitrogen). This mean is a little high for it includes the three days on which alkaline fermentation probably took place, and also the last two days when the ammonia excretion was high. Excluding these the average was 0.068 gram of nitrogen. The variations noted range from 0.039 gram to 0.085 gram of ammonia-nitrogen.

Creatinine.--The creatinine-nitrogen fell gradually from 0.032 gram on the first day to 0.012 gram on the twenty-sixth day, with slight variations which may be attributed to the method of urine collection (without catheterization). Unfortunately through accident we were prevented from obtaining creatine and creatinine data on the urine for the last three days of the fast. The excretion of nitrogen as creatinine decreased, from day to day, both absolutely and relatively. The maximum output was 2.96 per cent. on the first day, and the minimum 0.94 per cent. on the twenty-seventh day, of fast, the average being 1.85 per cent.

Creatine.--The creatine-nitrogen fell below that of the creatinine and remained fairly constant until the fifteenth day, when it became greater than the creatinine excretion, and remained above this value to the end of the fast. On the last two days, on which creatine was determined, there was a tendency toward a rise in the creatine-nitrogen excretion corresponding to the premortal rise. The average creatine excretion was 0.026 gram of nitrogen per day, the extremes being 0.008 gram on the fourteenth day, and 0.053 gram on the twenty-seventh day of fast. Relative to the total nitrogen the creatine-nitrogen excreted was an average of 2.05 per cent. with extremes of 0.67 per cent. on the fourteenth day and 3.64 per cent. on the twenty-sixth day.

Allantoin and Purine.-The allantoin- and purine-nitrogen did not show any marked tendency toward a definite variation. The average excretion was 0.005 gram of purine-nitrogen per day, and 0.004 gram of allantoin-nitrogen per day, which represents 0.38 per cent. of the total nitrogen as purine-nitrogen, and 0.31 per cent. as allantoin-nitrogen, respectively.

Undetermined Nitrogen.-The undetermined nitrogen was low, and practically constant in amount from day to day. It is interesting to note that the average daily amount of undetermined nitrogen in the second fast was only half that excreted during the first fast.

Discussion of the Two Fasts.

Data have been presented from two fasts upon a dog, which had previously never been subjected to an experiment of this character. The first fast continued for a period of fifteen days, or until the dog was in a state of collapse and the rise was in evidence. This fast was followed by an intermediate feeding period of forty-seven days, during which time the animal was restored to its original condition. Following this came the second fast, which continued for thirty days, or twice as long as the first fast.

Some important factors which affect the length or intensity of a fast are the race or breed, the age, the physical condition and activity, the surrounding temperature, the character of the preliminary feeding period, the general type of the nutritional and metabolic mechanism, and whether the fast is absolute or with water. Since the two fasts were to be compared, it was essential that the animal should be as nearly as possible in the same condition as regarded body weight, nitrogen equilibrium, etc., at the commencement of each fast, and, that the experimental procedure throughout the fasts should be similarly regulated. The body weight and nitrogen equilibrium were chosen as constants to be attained before the beginning of the fast, whereas the water ingested was

a constant for the whole experiment^{2.57}.

In this experiment breed and age do not enter directly. While the age of the animal affects the length of fast, in this case the dog was matured and the time between the two fasts was so short (47 days) that the age could not enter in any material way. Before each fast the dog was brought to a constant body weight, and was practically in nitrogen equilibrium. The body weight was a little greater at the beginning of the second fast, being 3.54 kilograms, while at the beginning of the first fast the weight was 3.41 kilograms. Whether this increase in weight was fat or muscular tissue will be discussed later, page A lower nitrogen balance was attained than before the first fast, + 0.02 gram of nitrogen against - 0.271 gram before the first fast. The physical condition of the animal immediately before the fasts was practically the same in the two instances. However, her coat was smoother and her muscles rather firmer at the commencement of the second fast.

Under the conditions outlined above, the dog experienced two fasts. That these were profound is evidenced by the presence of the premortal rise and the collapse of the animal in each case. The first fast was fifteen days long while the second was twice as long. These are seemingly short fasts, but considering the size and age of the dog they are of average length. The greater length of the second fast is probably due to the resistance of the tissues toward breakdown, acquired as a result of the first fast. During the first fast the tissues seem to have broken down very readily, so that the body lived improvidently upon its reserves. In the second fast, however, the protein and fat needed for the maintenance of life were used sparingly, and, as a consequence, the dog fasted longer. This low rate of catabolism may have occurred in one of two ways. The cells, as a result of the fast and the subsequent rebuilding, became more resistant, i. e. they gave up their protein constituents less readily to the blood stream. On the other hand, the amount of protein or

energy required for the functioning of the active organs, may have been lessened and consequently the latter did not draw upon the circulating protein as heavily as would have occurred had they not experienced a previous siege of fasting.

That the difference in the duration of the fasts was not a result of the amount of fat stored up in the body, can be shown from consideration of the nitrogen data and body weights. The calculations on this basis follow:-

Weight before first fast	3.41 Kg.
Weight after first fast	1.85 Kg.
Percentage loss in weight	45.76 o/o
Weight before second fast	3.54 Kg.
Weight after second fast	1.91 Kg.
Percentage loss in weight	46.04 o/o
Weight lost during first fast	1560 gr.
Nitrogen lost during first fast, 35.65 grams	
Equivalent of nitrogen in terms of flesh (3.25 per cent. N)	1100 gr.
Weight of fat and water lost (by difference)	460 gr.
Weight gained during feeding	1690 gr.
Nitrogen stored during feeding, 38.25 grams	
Equivalent of nitrogen in terms of flesh (3.25 per cent. N)	1180 gr.
Weight of fat and water stored (by difference)	510 gr.
Weight lost during second fast	1630 gr.
Nitrogen lost during second fast, 41.15 grams	
Equivalent of nitrogen in terms of flesh (3.25 per cent. N)	1260 gr.
Weight of fat and water lost (by difference)	370 gr.

When calculating all of the nitrogen lost as flesh, and the difference, between that and the loss in weight as fat, we see that during the feeding period the body increased its muscular tissue by 80 grams, and its fat by 50 grams, over the condition existing at the beginning of the first fast. We can not say positively that that which has been called fat in the above calculation is entirely fat, although the approximation is probably more accurate in the case of muscular tissue. However from a consideration of these data we may conclude that the dog was apparently in the same condition at the commencement of each fast.

The increased length of the second fast was not accompanied by a corresponding increase in the amount of muscular tissue and fat catabolized. There was an increase of about 5.5 grams in the total weight of nitrogen lost, which amount was lost during the last three days of the fast. The calculations for the second fast show that only 370 grams of fat were lost, while 510 grams were deposited during the intermediate feeding period, leaving 140 grams of this deposited fat in the body at the end of the fast. The fact that the post mortem examination showed that the animal was almost free from visible fat, would contradict this statement, unless it were in the form of combined fat (phosphatides) as suggested by MacLean and Williams⁵⁸.

Had the increased length of fast, and the decreased muscular catabolism been influenced by more extensive fat deposits a much larger amount of fat would have been used than can be accounted for. It seems therefore that the phenomena were due to the altered rate of the anabolic and catabolic activities of the tissues and organs involved.

Albitsky³ has shown that animals subjected to repeated fasting lose body weight less rapidly and further that the length of time necessary to secure a definite body weight loss is greater, than during the initial fast. A long series of repeated fasts conducted at short intervals without bringing the animal

back to the normal condition between fasts, may be accompanied by serious results, for Richet showed for example, that dogs fasted repeatedly for five days at a time lived only six months, and rabbits fasted at six day intervals lived even a shorter time.

Schulz¹² presents a theory for the explanation of the phenomena of decreased metabolism during interrupted feeding or repeated fasting. He suggests that when, as a result of the removal of the stimulation brought about by ingested food the activities of, what is probably the most important degree involved in metabolism, i. e., the liver, is impaired, and consequently the protein metabolism is led into abnormal paths and autointoxication may result. This phenomenon of autointoxication must be capable of being reduced upon the re-entrance of the stimulation of food. With the instigation of a new fast the condition of increased metabolism will not appear and as a result the injury resulting from the loss of the stimulation due to food will be very small, or will be entirely absent.

It is clear that such a theory takes no account of any metabolic activities other than those centered in the liver. While appreciating to the full the extremely important role played by this organ in the metabolic activities of the animal body, it nevertheless seems highly probable that other organs are involved in connection with the decreased protein catabolism of repeated fasting.

Schulz¹² theory, which would account for the collapse or the premortal rise as due to a defective liver function is not substantiated in the case of our dog, for it is evident that here the data would tend to show that there was a certain minimum amount of protein which must be present in the body in order that life shall exist. We believe this conclusion to be warranted from the fact that our data show that during each fast there were very similar amounts of nitrogen excreted notwithstanding one fast was twice as long as the other. For example during the first fast of fifteen days the dog lost 10.45 grams of

nitrogen per kilogram of body weight, whereas, during the second fast of thirty days she lost 11.60 grams of nitrogen per kilogram of body weight. In other words at the end of each fast there was evidently a similar residual quantity of nitrogen-holding substance remaining in the body, the nitrogen of which was not available for the metabolic uses of the organism.

Total Nitrogen.-The amount of nitrogen excreted in the urine in each fast was approximately the same, i. e. 34.689 grams in the first fast and 39.430 grams in the second fast. The greater absolute amount of nitrogen excreted during the second fast was due, partly to the increased amount of nitrogen stored during the intermediate feeding period (80 grams of muscular tissue, see page 24) and partly to the fact that the premortal rise of the first fast was incomplete. The average daily excretion was 2.313 grams of nitrogen for the first, and 1.314 grams of nitrogen for the second fast.

During the first fast the total nitrogen excretion increased very rapidly from day to day denoting a most pronounced acceleration of the disintegration of muscular tissue. After a drop for three days, a very marked premortal rise occurred on the last day, resulting in an excretion of 3.944 grams of nitrogen, which was 2.472 grams of nitrogen more than on the previous day. This increase was greater than the average daily excretion for the whole period.

The rapid loss of nitrogen during the first fast might be ascribed to one of two things: first, a lack of fat in the body, or second, that the dog was organically unsound. If our calculations hold good (page 24) there was more fat consumed during the first fast than during the second. The increased fat deposit (due to feeding) amounted to but 50 grams of fat, and was also accompanied by an increase in muscular tissue, and in body weight. It is evident that there must have been a good store of fat in the body before the first fast. This presumption was substantiated by the feel of the skin which denoted an ample subcutaneous fat deposit. Furthermore, the complete recovery of the dog

after the first fast, and the fact that this was followed by a subsequent fast of twice this length would denote an organically sound animal. The post-mortem examination substantiated the above conclusion.

These are general deductions but sufficiently strong to warrant the premise that the first fast was normal and the increased excretion of nitrogen was due to a rapid normal catabolism of the muscular tissues. This is further substantiated by the fact that more fat was consumed during the first fast than in the second, notwithstanding the fact that approximately equal amounts of nitrogen were excreted.

The rate of nitrogen excretion in the second fast was a marked contrast to that of the first fast. After the first day the excretion remained almost constant for twenty-five days (an average of 1.238 gram) when a gradual premortal rise set in. The excretion for the last sixteen hours being 2.298 grams of nitrogen. The low constant excretion during the second fast was that observed in most fasts, and accompanies the increased length of time over which the amount of available nitrogen was metabolised, since practically the same total amount of nitrogen was excreted in each fast.

Urea.-The nitrogen excretion as urea followed the total nitrogen excretion both absolutely and relatively. The average percentage of urea-nitrogen excretion was less in the second fast 86.51 per cent., or excluding the three days upon which alkaline fermentation seemed to have occurred, 87.46 per cent. In the case of the first fast the percentage excretion was 89.13 per cent. These values are higher than the average values for the normal diet. The fact that the ratio of urea-nitrogen to total nitrogen was constant has been discussed, see page 14; This constant ratio held for both fasts.

According to Folin's⁵⁰ theory the relative amount of urea excreted varies with the amount of total nitrogen. This law was enunciated with regard to normal metabolism. It has been shown in our laboratory²³ that with fasting men

the percentage of urea-nitrogen decreases, at least for a time. Schöndorf³⁵ and Underhill and Kleiner³⁴ hold this to be true for the dog. The latter investigators report a fourteen day fast, the data from which vary considerably, and the fluctuations in the percentage of urea-nitrogen do not agree with those of the total nitrogen.

In fasting man the decrease in the percentage of urea is probably due to the fatty acids⁶⁰ which by combining with the ammonia prevent the utilization of this ammonia in the formation of urea. The constant relative urea excretion in our experiment would tend to show that fatty acids or acid salts were probably not excreted in an increased amount during the fasting period.

There is a slight increase in the percentage of urea-nitrogen excreted during the first fast which would substantiate Folin's theory with regard to the effect of an increased nitrogen excretion. However, this point is more strikingly brought out in the case of the intermediate feeding period. An examination of Table III, page 62, will show that the percentage of total nitrogen in the form of urea increased from 32.67 per cent. to 86.51 per cent., then to 87.40 per cent. and finally to 88.74 per cent. as the diet was progressively increased.

It appears therefore that in a fasting dog the relative urea excretion follows in general the laws of relative urea excretion which obtain for normal feeding. For, with an increased urea excretion, as in the first fast, there is a slight increase in the relative urea-nitrogen excretion, and with a low and constant total nitrogen excretion in the second fast, there is a lower and more constant relative urea excretion. Why this should hold for the dog and not for man is not clear. It may be perhaps that the dog, being a carnivorous animal, and living on the same form of food in each case (fresh lean meat, and muscular tissue) excretes in each instance the same percentage of total nitrogen in the form of urea. Man on the other hand, being unaccustomed to a diet of uncooked

lean meat finds the conditions unusual when forced to exist upon his own tissues during a fasting test, and consequently under these conditions a different quota of the total nitrogen output is eliminated as urea, than when he ingests the normal mixed diet.

Ammonia.-The ammonia nitrogen excretion was less for the daily average in the second fast, 0.083 gram or, excluding the questionable days, already mentioned, 0.068 gram of nitrogen per day, as against 0.103 gram of nitrogen in the first fast. Instead of increasing from day to day, as in the fasts of men, the ammonia remained fairly constant, following the total nitrogen excretion, as Folin⁵⁶ has pointed out, only in that when the total nitrogen excretion was low, the ammonia was absolutely lower, but relatively higher. A rise in the ammonia excretion at the end corresponded in each case with the premortal rise. The average ratio of ammonia-nitrogen to total nitrogen was, in the first fast, 4.59 per cent., and, in the second fast, 6.12 per cent., or, excluding the days of alkaline fermentation 5.66 per cent., which shows the relatively higher ammonia excretion during the second fast. The facts noted here with regard to the ammonia excretion are those which hold in normal feeding.

Creatinine.-The marked differences in the total nitrogen excretions were not accompanied by similar changes in the creatinine-nitrogen output. In both fasts the creatinine-nitrogen fell gradually, the average daily excretion for each period being almost the same, that for the first fast being 0.025 gram of nitrogen and for the second 0.023 gram. The total amount excreted in the second fast was nearly twice as great as that of the first fast, the output of creatinine-nitrogen, therefore, varying inversely as the length of the fast.

Folin⁵⁶ first enunciated the law that the amount of creatinine excreted in the urine by a normal individual is quite independent of either the amount of protein in the food, or the total nitrogen in the urine. This has been confirmed by van Hoogenhuyze and Verploegh⁴⁰, Klerker⁴¹, Closson⁴², Shaffer⁴³. Cathcart⁴⁴

and Paton⁶⁵ fail to verify Folin's theory. Folin has further pointed out that the chief factor determining the amount of creatinine eliminated appears to be the weight of the individual, and that it also depends upon the mass of active protoplasmic tissue. Shaffer⁶³ virtually substantiates this theory. The creatinine elimination in fasting men has been observed by van Hoogenhuyze and Verploegh, Benedict⁴¹, Benedict and Diefendorf⁴² and Cathcart⁶⁶, and in the fasting dog, by Underhill and Kleiner³⁴, and Howe, Mattill and Hawk²³, a gradual decrease having been observed in each case Underhill and Kleiner state that the creatinine excretion is variable. Shaffer lays stress upon the creatinine coefficient (milligrams per kilogram body weight) as a constant for individuals of an equal muscular efficiency, which should then decrease for a decreasing muscular efficiency.

The data from our experiment confirm, in the main, these theories. With a decreasing body weight (loss of muscular tissues) and muscular efficiency there is a decrease in the amount of creatinine excreted and also in the creatinine coefficient, Table V, page 70. The amount of creatinine excreted, per day, bears a direct relation to the amount of active muscular tissue present in the body. The creatinine excreted in our experiment does not bear any direct relation to the nitrogen excretion, for we have a similar excretion of creatinine in the two fasts, notwithstanding the fact that the rate of the nitrogen excretion was markedly different in the two cases and that the average daily excretion of total nitrogen was nearly 100 per cent. greater in the first fast than in the second fast.

That it is the decrease in the amount of active protoplasm, and not merely the body weight, which causes a decrease in the creatinine excretion may be seen from an examination of the data relative to the creatinine coefficient. If the decrease were coincident with the decrease in weight the creatinine coefficient would remain constant, whereas in reality it decreases more slowly than the

body weight. This dependence upon the amount of muscular tissues is shown in the intermediate feeding period. As the dog gained in weight and stored nitrogen the amount of creatinine increased, although it did not bear any direct relation to the amount of nitrogen excreted. On the nineteenth day or at the time 34 per cent. of the body weight lost in the fast, and one-fourth the nitrogen lost, had been restored, the creatinine coefficient was nearly equal to that before the fast. Furthermore on the twenty-fifth day or at the time the animal had regained 50 per cent. of its lost nitrogen and body weight, the creatinine coefficient was the same as that secured before the fast when the dog was of constant body weight and in nitrogen equilibrium. If current ideas regarding this coefficient are correct the findings indicate that the animal possessed equal muscular efficiency under the two conditions.

The above may be substantiated by the following data:-

Total amount of nitrogen lost during first fast	34.649 grms.
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Creatinine coefficient before fast = 12 mgs.

Nitrogen stored during 19 days of feeding (creatinine coefficient 11 mgs.) and 34 per cent. gain in body weight.	9.015 grms.
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Nitrogen stored during 25 days of feeding (creatinine coefficient 12 mgs.) and 50 per cent. gain in body weight.	17.157 grms.
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We thus see that by the time the original creatinine coefficient was restored, i. e. on the twenty-fifth day of feeding the mass of muscle was only 50 per cent. of that present before the fast. If we are to hold to the idea that the ingested creatine (in the meat) and the amount of nitrogen excreted have no effect upon the creatinine output, then in order that the creatinine coefficient shall be significant, we must suppose a return to the original condition as regards muscular efficiency when the animal had only restored one-half of its nitrogen and body weight. The dog certainly, at this point in the experiment, ex-

hibited all of her normal activity which fact tends to confirm the above remarks with regard to muscular efficiency.

Creatine.-The excretion of creatine in the two fasts was quite similar, being marked by a drop in the creatine-nitrogen excretion at the beginning of the fast, followed by a period during which the creatine-nitrogen remained fairly constant, and a little less than the creatinine excretion. Toward the end of the fast, and before the premortal rise there was a sudden increase in the amount of creatine-nitrogen excreted, being greater than the creatinine excretion. This increased creatine continued to the end. This marked increase of the creatine excretion over the amount of creatinine excreted seems to be an indication of the pronounced metabolic irregularities which finally result in the inception of the premortal rise, and occurs from two to three days before the drop in the total nitrogen excretion, which preceeds this rise.

The amount of creatine excreted in the two fasts is almost equal, corresponding to the amount of total nitrogen excreted during the periods in which creatine was determined. Benedict⁴¹, Mellanby⁴⁸, Shaffer⁴³, Paton⁴⁵, Fowler and Hawk⁴⁷ and others have furnished experimental evidence in support of the theory that the creatine in the urine arises as a result of the breakdown of the muscular tissues, Paton suggested that the discrepancy between the amount of flesh which was catabolized as calculated from the creatine output and that calculated from the total nitrogen, may be due to the resynthesis of a part of the nitrogen of the broken down muscular tissue, the creatine being excreted at the time of the muscle breakdown.

Calculating upon the basis of the excretions of total nitrogen and creatine-nitrogen we have derived in each case the weight of the mass of tissue catabolized assuming the accuracy of the theory that urinary creatine represents disintegrated muscular tissue. The data are appended:-

Flesh Lost as Calculated from the Urinary Total Nitrogen
Data and from the Creatine-Nitrogen Data.

First Fast (15 days)

Nitrogen lost - 34.689 grams.

Equivalent in terms of flesh - (3.25 o/o) = 1069 grams

Creatine-nitrogen lost - 0.684 gram

Equivalent in terms of flesh (0.123 o/o) = 555 grams

Second Fast (27 days)

Nitrogen lost - 33.524 grams.

Equivalent in terms of flesh-(3.25 o/o) = 1030 grams.

Creatine-nitrogen lost - 0.689 gram.

Equivalent in terms of flesh - (0.123 o/o) = 560 grams.

The amount of creatine-nitrogen present in the muscles of the dog at the end of the fast was 0.042 per cent., showing a very marked decrease (66 per cent.) in the amount of creatine in the fasting muscle remaining in the body. Dorner has reported a somewhat smaller decrease in the case of rabbits' muscle. His data show the amount of creatine in this tissue to be reduced from 0.456 per cent. to 0.356 per cent. as a result of fasting. This pronounced decrease of creatine found by us in fasting muscle is a most significant fact and shows clearly that in fasting we cannot with accuracy consider the total amount of excreted creatine as resulting from the complete and permanent disintegration of muscular tissue. However, even if we do consider all of the excreted creatine as having this origin we have shown by calculation that we can account for only about one-half of the total nitrogen on this basis. The discrepancy between the mass of muscular tissue lost, calculated on the two bases, i. e., total nitrogen and creatine-nitrogen output becomes all the more striking when we appreciate that a large part of the creatine excreted during the fasts and which is ordina-

rily considered as representing completely disintegrated muscular tissue, in reality most certainly does not represent this but rather has been withdrawn from muscular tissue which is still functioning as living tissue within the body of the animal. Unfortunately no determination was made of the actual mass of muscular tissue remaining in the body after fasting. However inasmuch as we have shown in this laboratory⁷⁰ that the total nitrogen content of fasting muscle is only slightly lower than that of well nourished muscle it is evident that but a small fraction of the total nitrogen excreted by the urine arose from the muscular tissue still functioning within the body whereas a comparatively large part of the creatine had this origin. When all these facts are taken into consideration it is apparent that over 50 per cent. of the total nitrogen of the urine had a source other than the catabolism of muscular tissue. The fact that fasting muscle may have its creatine store depleted to one-third the normal while retaining its nitrogen content but slightly lowered indicates rather conclusively that the creatine is held in such a combination within the muscle that its releast is not necessarily accompanied by the disintegration of the other nitrogenous muscle constituents which go to form the total nitrogen of muscular tissue^{68,57}. At the same time we must not lose sight of the fact that the muscular tissue whose nitrogen store is seemingly intact at the end of a fast may assume this condition not because the catabolic processes have not been sufficiently profound to alter its inner structure but rather because of certain synthetic reactions which have followed or accompanied the primary disintegration of the tissues⁶⁵. That there is a direct relation between the amount of nitrogenous material catabolized and the creatine excreted is brought out in our results. For example, with practically the same amount of nitrogen excreted under the same conditions, i. e., two fasts continuing to the premortal rise, and with two equal but decidedly different rates of nitrogen excretion, practically the same amount of creatine was excreted.

No direct evidence has been obtained which would show the relation between creatine and creatinine. The creatine appears to be derived either from disintegrating muscular tissue or to be removed in some manner from such tissues which are still functioning within the body. The creatine excretion was low during the first part of each fast increasing suddenly a few days before the drop in the total nitrogen excretion which preceded the premortal rise. From this time to the end of the fast the daily output of creatine-nitrogen exceeded that of the creatinine-nitrogen. Dörner²⁹ in the case of a rabbit which died after nine days of fasting found the creatine excretion of the second day to exceed that of creatinine.

SUMMARY.

A fox terrier bitch, about one year old and weighing 3.41 kilograms was brought into nitrogen equilibrium and was subjected to two fasts. On the fifteenth day of the first fast the premortal rise in nitrogen output was noted and was accompanied by other signs indicating that death would result in a few hours. She was then carefully fed and during the feeding period of forty-seven days regained her former weight and was again brought into nitrogen equilibrium, after which she was fasted a second time for a period of thirty days. The animal was in fully as good physical condition at the beginning of the second fast as she was at the commencement of the first fast.

The water ingestion was uniform (250 cc.) throughout the feeding and fasting periods. The loss in body weight was nearly equal in each fast being 45.75 per cent. for the first fast and 46.04 per cent for the second fast. However on the fifteenth day of the second fast i. e., after a period equalling in length the entire first fast, the animal had lost but 25.42 per cent. ^{of} her body weight.

From the data obtained we have shown that:-

1. With a practically equal total nitrogen excretion, the rate of this excretion was widely different in the two fasts, being high and rapidly increasing during the first fast, of fifteen days, and low and fairly constant during the second fast of thirty days. A premortal rise occurred at the end of each fast.

2. The absolute amount of urea-nitrogen excreted was in direct relation to the total nitrogen excretion. The relative urea-nitrogen excretion remained practically constant throughout the fasts, but increased with an increased total nitrogen excretion during the intermediate feeding period.

3. The ammonia-nitrogen excretion remained fairly constant, ⁴var~~ing~~ with the total nitrogen excretion in that when this was low the ammonia-nitrogen was absolutely lower but relatively higher.

4. The creatinine-nitrogen excretion decreased very uniformly and gradually as the fasts progressed, and increased uniformly and gradually during the intermediate feeding period. The total output of creatinine-nitrogen varied inversely as the length of the fast. On the nineteenth day of the feeding period, or at the time thirty-four per cent. of the body weight lost in the first fast, and one-fourth the nitrogen lost, had been restored, the creatinine coefficient was nearly equal to that before the fast. Furthermore on the twenty-fifth day or at the time the animal had regained only fifty per cent. of its lost nitrogen and body weight the creatinine coefficient was the same as that secured before the fast when the dog was of constant body weight and in nitrogen equilibrium.

5. The total amount of creatine-nitrogen excreted was practically the same during each fast. The average daily amount of creatine-nitrogen, however, was nearly twice as great during the first fast as during the second fast.

6. The allantoin- and purine-nitrogen excretions decreased as a result of the fast and were practically equal for each fast.

7. The undetermined nitrogen decreased as a result of fasting. The average daily amount of undetermined nitrogen in the second fast being one-half that in the first fast.

8. A consideration of the summation of the nitrogen balances shows that there is a minimum amount of nitrogen which must be present in the body in order that life shall exist.

9. The excretion of urinary creatine increased suddenly a few days before the drop in the total nitrogen excretion which preceeds the premortal rise. From this time to the end of the fast the daily output of creatine-nitrogen exceeded that of the creatinine-nitrogen.

10. Assuming the accuracy of the theory that the urinary creatine represents disintegrated muscular tissue and calculating accordingly a discrepancy exists between the calculated mass of muscular tissue lost, when considered from the standpoint of total nitrogen and creatine-nitrogen respectively. When all the facts in this connection are taken into consideration it is apparent that over fifty per cent. of the total nitrogen had a source other than the muscular tissue.

11. The creatine content of muscle showed a marked decrease (over sixty per cent.) as a result of fasting, while the nitrogen content of similar muscle was but slightly lowered. This pronounced decrease of creatine found by us in fasting muscle is a most significant fact and shows clearly that in fasting we can not with accuracy consider the total amount of excreted creatine as resulting from the complete and permanent disintegration of muscular tissue. It appears that the creatine of the urine is derived either from disintegrating muscular tissue or is removed in some manner from such tissues which are still functioning within the body.

12. As a result of our experiments we have shown that in repeated fasting there is a slower and less profound tissue disintegration during the second

fast, indicating a greater resistance of the body, acquired as a result of the initial fast. The greater length of the second fast could not have been due to increased fat stores, for the fat present in the body of the animal at the beginning of the second fast was, according to our calculations, only fifty grams more than that at the beginning of the first fast.

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Biographical.

Paul Edward Howe graduated from the University of Illinois in 1906 with the degree of Bachelor of Science in Chemistry. He received a scholarship in chemistry, in the University of Illinois, for the year 1906 to 1907 and obtained the degree of Master of Arts in 1907 with a thesis in physical chemistry from that institution. During the following year he was assistant chemist in the Laboratory of Physiological Chemistry of the University of Illinois and did graduate work in physiological chemistry. As assistant in physiological chemistry in the University of Illinois he pursued graduate courses in physiological chemistry under Professor Philip B. Hawk and in physiology and histology.

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TABLE I
General Data.

Day of Expt.	Body Weight Kgs.	Volume of Urine c.c.	Specific Gravity	Reaction of Urine	Total N grams	Urea N grams	Ammonia N grams	Creatinine N grams	Creatine N grams	Purine N grams	Allantoin N grams	Undetermined N grams
Preliminary Feeding Period												
1	3.50	270	10125		2.707	2.354	0.104	0.051	0.008			0.190
2		256	1013	acid	2.202	1.940	0.092	0.037	0.079		0.002	0.032
3		270	10145	"	2.630	2.279	0.122	0.040	0.033		0.003	0.153
4	3.46	265	1012	sl. acid	2.580	2.240	0.118	0.038	0.052	0.015	0.004	0.113
5		260	1013	amm.	2.555	2.168	0.145	0.036	0.013	0.006	0.023	0.164
6	3.42	240	1014	"	2.964	2.524	0.205	0.043	0.074	0.016	0.010	0.092
7	3.40	310	1014	acid	3.683	3.312	0.156	0.040	0.121		0.009	0.046
8	3.41	245	10105	"	2.825	2.425	0.206	0.041	0.074			0.079
First Fast												
1	3.26	35	10115	acid	2.634	2.305	0.118	0.060	0.037	0.006	0.013	0.095
2	3.19	227		"								
3	3.13	235	10055	"	1.489	1.293	0.093	0.029	0.021			0.053
4	[3.04]	188	1011	"	1.960	1.755	0.087	0.032	0.032			0.054
5	2.95	219	10125	"	2.250	2.020	0.132	0.036	0.023	0.012	0.008	0.029
6	2.91	255	10065	"	1.862	1.608	0.113	0.023	0.030			0.076
7	2.86	164	1014	"	2.558	2.331	0.102	0.029	0.026			0.070
8	2.77	288	10075	"	2.308	2.037	0.118	0.026	0.037	0.001	0.001	0.088
9		260	1010	"	2.637	2.399	0.096	0.026	0.054	0.006		0.056
10	2.60	267	10095	"	2.780	2.502	0.102	0.026	0.062	0.006	0.002	0.080
11	2.49	262	10095	"	2.777	2.495	0.105	0.024	0.075	0.012	0.004	0.062
12	2.31	292	10085	"	2.483	2.208	0.109	0.019	0.077	0.001	0.002	0.067

TABLE I (cont.)

First Fast (cont.)

13	2.22	280	1009	acid	2.535	2.261	0.105	0.015	0.071	0.004	0.004	0.075
14	2.05	283	1009	"	2.472	2.214	0.110	0.012	0.069	0.007	0.007	0.053
15	1.85	325	1013	"	3.944	3.544	0.160	0.016	0.070		0.012	0.142

Feeding Periods

Preliminary

1	1.95	215	1011	acid	2.893	2.633	0.104	0.010	0.044			0.116
2	2.25	50	10145	"	1.670	1.377	0.117	0.025	0.050	0.007	0.008	0.086
3	2.35	261		"								
4	2.24	443	1006	"	0.860	0.641	0.095	0.014	0.022	0.004	0.008	0.076

Normal Diet

5	2.20	250	1008	alk.	1.356	1.160	0.079	0.013	0.036			0.061
6	2.12	258	1008	"	1.840	1.496	0.211	0.017	0.043			0.066
7	2.19	241	10075	"	1.716	1.465	0.119	0.016	0.015	0.029	0.004	0.094
8	2.24	250	1008	acid	1.595	1.381	0.101	0.018	0.041			0.047
9	2.25	238	1007	alk.	1.575	1.406	0.078	0.018	0.048			0.018
10	2.26	257	1008	"	1.835	1.599	0.087	0.021	0.065			0.057
11	2.27	269	1009	"	1.929	1.678	0.101	0.020	0.061	0.024		0.063
12	2.29	250	1009	"	1.900	1.659	0.100	0.018	0.047			0.070
13	2.28	258	10085	"	1.952	1.717	0.100	0.027	0.071			0.075
14	2.31	198	1010	"	1.515	1.319	0.077					0.051
15	2.34	100	1011	"	4.368	3.740	0.289	0.048	0.127	0.018	0.029	0.133
16	2.36	385	1011	amph.								
17	2.36	235	1010	acid	3.889	3.433	0.204	0.044	0.082			0.088
18	2.36	265	10105	amph.						0.028	0.050	
19	2.38	240	1009	"	2.030	1.750	0.140	0.026	0.058			0.037

TABLE I (con)

Day	Body wt	Vol.	Sp.gr.	Reaction	Total N	Urea N	NH ₃ N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter. N
50% Increase in Diet												
20	2.42	212	1013	amph.	2.594	2.236	0.179	0.026	0.079			0.055
21	2.49	235	10125	"	5.545	4.837	0.228	0.052	0.191			0.135
22	2.50	295	10125	acid						0.026	0.075	
23	2.57	250	1013	"	5.535	4.848	0.283	0.056	0.203			0.094
24	2.61	265	1013	"								
25	2.63	248	10125	sl. acid	5.183	4.547	0.276	0.061	0.219	0.014	0.023	0.040
26	2.67	225	1014	"								
27	2.75	232	1012	acid	2.655	2.343	0.110	0.035	0.120	0.009	0.006	0.032
100% Increase in Diet												
28	2.84	267	1017	sl. acid	3.682	3.250	0.152	0.035	0.120	0.009	0.006	0.110
29	2.85	304	10145	amph.	7.858	7.010	0.344	0.068	0.246		0.029	0.161
30	2.92	235	10175	sl. acid								
31	3.00	215	1017	acid	7.286	6.380	0.325	0.072	0.269		0.020	0.220
32	3.00	255	10175	"								
33	3.09	250	1020	sl. acid	8.577	7.515	0.406	0.076	0.241		0.015	0.324
34	3.10	275	10195	amph.								
35	3.20	220	1020	acid	7.606	6.580	0.378	0.076	0.283	0.022	0.016	0.232
36	3.24	230	1021	alk.								
37	3.32	265	1020	acid	8.560	7.533	0.357	0.086	0.290			0.254
38	3.35	258	10185	"								
39	3.40	211	1023	amph	8.283	7.275	0.387	0.082	0.276	0.131	0.032	0.223
40	3.46	215	10225	"								

TABLE I (con)

100% (con)									
41	3.50	212	1024	amph.	8.762	7.695	0.355	0.089	0.258
42	3.52	260	1019	acid					0.325
43	3.56	220	10215	"	8.592	7.550	0.351	0.089	0.298
44	3.59	240	1023	amph.					0.262
Normal Diet									
45	3.56	200	1014	acid	4.920	4.290	0.228	0.082	0.170
46	3.54	250	1012	"					0.024
47	3.54	224	1013	"	2.435	2.105	0.111	0.039	0.046
Second Fast									
1	3.42	230	10055	acid	1.081	0.941	0.046	0.032	0.029
2	3.32	252	1005	"	1.197	1.036	0.058	0.032	0.031
3	3.26	212	1005	"	1.209	1.058	0.052	0.030	0.014
4	3.11	295	10075	alk	1.202	1.059	0.039	0.034	0.028
5	3.07	216	10045	acid	1.251	1.086	0.062	0.031	0.032
6	2.94	66	1023	"	1.471	1.317	0.051	0.034	0.026
7	[3.05]	122	10085	"	1.128	0.985	0.057	0.027	0.018
8	2.95	248	1005	"	1.368	1.209	0.070	0.026	0.024
9	2.89	255	1004	"	1.218	1.063	0.074	0.025	0.016
10	2.86	214	1005	"	1.194	1.056	0.075	0.026	0.012
11	2.80	241	10035	"	1.158	1.026	0.070	0.024	0.014
12	2.77	204	10045	"	1.332	1.173	0.078	0.027	0.019
13	2.72	231	1006	amph.	1.357	1.192	0.085	0.025	0.014
14	2.68	210	1005	"	1.182	1.028	0.083	0.024	0.008
15	2.64	209	1005	acid	1.207	1.097	0.084	0.023	0.016

TABLE I (concluded)

Day	Body wt.	Vol.	Sp-gr.	Reaction	Total N	Urea N	NH ₃ N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter. N
Second Fast (con)												
16	2.58	233	1005	acid	1.344	1.183	0.066	0.020	0.023			0.048
17	2.51	232	1004	alk.	1.119	0.879	0.167	0.018	0.018			0.033
18	2.49	197	1005	amph.	1.193	1.025	0.085	0.021	0.025			0.033
19	2.45	231	10045	alk + sl. acid	1.335	1.094	0.138	0.020	0.035			0.041
20	2.40	224	1004	acid	1.257	1.107	0.062	0.018	0.036	0.026	0.010	0.027
21	2.37	215	1004	"	1.221	1.056	0.067	0.016	0.035			0.040
22	2.36	215	1005	"	1.203	0.921	0.076	0.014	0.038			0.048
23	2.31	260	10055	amph alk	1.315	1.079	0.139	0.013	0.033			0.042
24	2.21	246	10045	acid	1.216	1.071	0.082	0.012	0.024			0.018
25	2.18	217	1004	"	1.145	1.003	0.074	0.012	0.027	0.029	0.017	0.020
26	2.15	215	1004	"	1.126	0.971	0.060	0.012	0.041			0.033
27	2.11	232	1005	"	1.495	1.318	0.065	0.014	0.053			0.036
28	2.05	252	10055	"	1.519	1.329	0.083					
29	1.97	2.71	1007	"	2.089	1.826	0.153					
30	1.91	198	1015	alk.	2.298	2.036	0.182					

TABLE II
Percentage Nitrogen Distribution

Day	Urea N	NH ₃ N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter N
Preliminary Feeding Period							
1	86.96	3.84	1.88	0.295			0.700
2	88.10	4.18	1.68	3.59		0.09	1.45
3	86.65	4.64	1.52	1.25		0.114	5.82
4	86.82	4.57	1.47	2.01	0.581	0.155	4.38
5	84.85	5.67	1.41	0.509	0.24	0.90	6.42
6	85.15	6.92	1.45	2.49	0.54	0.34	3.10
7	89.93	4.24	1.08	3.28		0.24	1.25
8	85.84	7.29	1.45	2.62			2.79
First Fast							
1	87.51	4.48	2.28	1.40	0.23	0.49	3.60
2							
3	86.84	6.24	1.95	1.41			3.56
4	89.54	4.44	1.63	1.63			2.75
5	89.78	5.87	1.60	1.02	0.27	0.18	1.29
6	86.36	6.07	1.23	1.61	0.32	0.21	4.08
7	91.12	3.99	1.13	1.02			2.74
8	88.26	5.11	1.13	1.60	0.04	0.04	3.81
9	90.97	3.64	0.98	2.05	0.23		2.12
10	90.00	3.67	0.93	2.23	0.22	0.07	2.88
11	89.84	3.78	0.86	2.70	0.43	0.14	2.23
12	88.92	4.39	0.76	3.10	0.04	0.08	2.70
13	89.19	4.14	0.59	2.80	0.16	0.16	2.96
14	89.56	4.45	0.48	2.79	0.28	0.28	2.14
15	89.86	4.06	0.46	1.77		0.30	3.60
Intermed. Preliminary Feeding							
1	91.01	3.59	0.34	1.52			4.01
2-3	82.45	7.00	1.50	2.99	0.42	0.48	5.15
4	74.53	11.05	1.63	2.56	0.46	0.93	8.84

TABLE II (con)

Day	Urea N	NH ₃ N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter N
Normal Diet							
5	85.55	5.82	0.96	2.65	0.44	0.07	4.50
6	81.30	11.46	0.92	2.34	0.33	0.05	3.59
7	85.37	6.93	0.93	0.87	0.35	0.06	5.48
8	86.58	6.33	1.13	2.57	0.38	0.06	2.95
9	89.27	4.95	1.14	3.05	0.38	0.06	1.14
10	87.14	4.74	1.14	3.54	0.33		3.11
11	86.99	5.23	1.04	3.16	0.31		3.27
12	87.31	5.26	0.94	2.47	0.32		3.68
13	87.96	5.12	0.78	1.84	0.31		3.84
14	87.06	5.08		2.31	0.40	0.59	3.37
15-16	85.62	6.61	1.10	2.91	0.28	0.43	3.04
17-18	88.27	5.24	1.12	2.11	0.36	0.64	2.26
19	86.21	6.90	1.28	2.86	0.35	0.59	1.82
50% Increase in Diet							
20	86.21	6.90	1.00	3.04	0.27	0.46	2.12
21-22	87.23	5.19	0.94	3.44	0.23	0.69	2.43
23-24	87.59	5.11	1.01	3.67	0.24	0.69	1.61
25-26	87.73	5.32	1.17	4.23	0.27	0.44	0.77
27	88.25	4.14	1.32	4.52	0.34	0.23	1.21
100% Increase in Diet							
28	88.25	4.13	0.95	3.26	0.24	0.16	2.99
29-30	89.21	4.38	0.86	3.13		0.37	2.05
31-32	87.56	4.46	0.99	3.69		0.28	3.02
33-34	87.62	4.73	0.88	2.81		0.18	3.78
35-36	86.51	4.97	1.00	3.72	0.28	0.21	3.05
37-38	88.02	4.17	1.00	3.39	0.37	0.05	2.97
39-40	87.83	4.67	0.99	3.33	0.39	0.05	2.69
41-42	87.82	4.05	1.01	2.94	0.37	0.05	3.71
43-44	87.87	4.08	1.03	3.47	0.37	0.05	3.05
Normal Diet							
45-46	87.19	4.63	1.67	3.46	0.33	0.20	2.72
47	86.44	4.56	1.60	1.89	0.33	0.21	4.97

TABLE II (concluded)

Day	Urea N	NH ₃ N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter N
Second Fast.							
1	87.05	4.25	2.96	2.68		0.28	2.78
2	86.54	4.84	2.67	2.59		0.25	3.09
3	87.51	4.30	2.48	1.16	0.33	0.41	3.80
4	88.10	3.25	2.82	2.33	0.33	0.42	2.74
5	86.81	4.95	2.48	2.56	0.32	0.40	2.48
6	89.53	3.46	2.31	1.77	0.27	0.34	2.31
7	87.32	5.05	2.39	1.60	0.36	0.44	2.84
8	88.38	5.12	1.90	1.75	0.29	0.37	2.19
9	87.27	6.07	2.05	1.31	0.33	0.41	2.38
10	88.44	6.28	2.18	1.01	0.34	0.42	1.34
11	88.60	6.03	2.07	1.21	0.35	0.43	1.29
12	88.06	5.85	2.02	1.43	0.30	0.38	1.95
13	87.84	6.26	1.84	1.03		0.30	2.73
14	86.97	7.02	2.03	0.68		0.34	2.96
15	90.88	6.96	1.90	1.32		0.33	
16	88.02	4.91	1.49	1.71		0.30	3.57
17	78.54	14.92	1.61	1.61		0.36	2.95
18	85.92	7.12	1.76	2.10		0.34	2.77
19	81.95	10.30	1.50	2.62	0.37	0.15	3.07
20	88.07	4.93	1.43	2.86	0.40	0.16	2.15
21	86.49	5.49	1.31	2.87	0.41	0.16	3.27
22	76.56	6.32	1.16	3.16	0.42	0.17	12.36?
23	82.05	10.57	0.99	2.51	0.46	0.23	3.19
24	88.08	6.74	0.98	1.97	0.49	0.25	1.48
25	87.59	6.46	1.05	2.36	0.52	0.26	1.76
26	86.23	5.33	1.06	3.64	0.53	0.27	2.93
27	88.16	4.35	0.94	3.54	0.40	0.20	2.41
28	87.49	5.46					
29	87.41	7.33					
30	88.59	7.92					

TABLE III
Summary of Nitrogen Excretion

Period (Diet)	Total N	Urea N	Ammonia N	Creatinine N	Creatine N	Purine N	Allantoin N	Undeter N.
Period Averages - Grams per Day.								
Preliminary	2.768	2.405	0.143	0.041	0.056	0.012	0.008	0.109
First Fast	2.313	2.065	0.103	0.025	0.046	0.005	0.005	0.067
Prelim. Feed.	1.356	1.162	0.105	0.012	0.029			0.070
Normal	1.833	1.587	0.112	0.020	0.054			0.076
50%	2.689	2.351	0.142	0.028	0.102	0.008	0.008	0.045
100%	4.071	3.576	0.178	0.040	0.134			0.124
Normal	2.452	2.132	0.113	0.040	0.072			0.085
Second Fast.	1.314	1.141	0.083	0.023	0.026	0.005	0.004	0.032
Period Averages - Percent.								
Preliminary		86.79	5.17	1.49	2.61	0.45	0.31	4.03
First Fast.		89.13	4.59	1.14	1.94	0.22	0.26	2.89
Prelim. Feed		82.67	7.21	1.11	2.36	0.45	0.71	6.06
Normal		86.51	5.69	1.65	2.51	0.35	0.28	3.25
50%		87.40	5.33	1.09	3.78	0.27	0.50	1.63
100%		88.74	4.40	0.97	3.30	0.46	0.15	3.03
Normal		86.81	4.59	1.63	2.68	0.33	0.20	3.56
Second Fast.		86.51	5.66	1.85	2.05	0.38	0.31	2.58
Total Weight of Nitrogen Excreted in Urine (grams)								
First Fast.	34.689	30.972	1.550	0.373	0.684			
Second Fast	39.430	34.224	2.483	0.611	0.689			

TABLE IV
NITROGEN BALANCE

Day	N in Urine grams	N in Hair, Feces and Cage Wash grams.	Total N Output grams	N in Food grams	N Balance	
Preliminary Feeding Period						
1	2.707	0.235	2.942	2.732	-0.210	
2	2.202	"	2.437	"	+0.295	
3	2.630	"	2.865	"	-0.133	
4	2.580	"	2.815	"	-0.083	
5	2.555	"	2.790	"	-0.058	
6	2.964	"	3.199	"	-0.467	
7	3.683	"	3.918	"	-1.186	
8	2.825	"	3.060	"	-0.328	
First Fast						
1	2.634	0.064	2.698		-2.698	
2						
3	1.489	"	1.653		-1.553	
4	1.960	"	2.024		-2.024	
5	2.250	"	2.314		-2.314	
6	1.862	"	1.926		-1.926	
7	2.558	"	2.622		-2.622	
8	2.308	"	2.372		-2.372	
9	2.637	"	2.701		-2.701	
10	2.780	"	2.844		-2.844	
11	2.777	"	2.841		-2.841	
12	2.483	"	2.547		-2.547	
13	2.535	"	2.599		-2.599	
14	2.472	"	2.536		-2.536	
15	3.944	"	4.008		-4.008	
Preliminary Feeding						
1	2.893	0.161	3.054	0.198	-2.856	
2	1.670	"	1.992	0.792	-0.144	
3		"		1.056		
4	0.860	"	1.021	2.239	+1.218	

TABLE IV (con.)

Day	N in Urine	N in Hair-Faces and Cage Wash	Total N Output	N in Food	N Balance	
Normal Diet						
5	1.356	0.161	1.517	2.465	+0.948	
6	1.840	"	2.001	2.732	+0.731	
7	1.716	"	1.877	"	+0.855	
8	1.595	"	1.756	"	+0.976	
9	1.576	"	1.737	"	+0.995	
10	1.835	"	1.996	"	+0.736	
11	1.929	"	2.090	"	+0.642	
12	1.900	"	2.061	"	+0.671	
13	1.952	"	2.113	"	+0.619	
14	1.515	"	1.676	"	+1.056	
15-16	4.368	0.322	4.690	5.464	+0.774	
17-18	3.889	"	4.211	"	+1.253	
19	2.030	0.161	2.191	2.732	+0.541	
50% Increase in Diet						
20	2.594	0.247	2.841	4.104	+1.263	
21-22	5.545	0.494	6.039	8.208	+2.169	
23-24	5.535	0.494	6.029	8.208	+2.179	
25-26	5.183	0.494	5.677	8.208	+2.531	
27	2.655	0.247	2.902	4.104	+1.202	
100% Increase in Diet						
28	3.682	0.256	3.938	5.464	+1.526	
29-30	7.858	0.512	8.370	10.928	+2.558	
31-32	7.286	"	7.798	"	+3.130	
33-34	8.577	"	9.089	"	+1.839	
35-36	7.606	"	8.118	"	+2.810	
37-38	8.560	"	9.072	"	+1.856	
39-40	8.283	"	8.795	"	+2.133	
41-42	8.762	"	9.274	"	+1.654	
43-44	8.592	"	9.104	"	+1.824	
Normal Diet						
45-46	4.920	0.634	5.554	5.464	-0.090	
47	2.435	0.317	2.752	2.732	+0.020	

TABLE IV (concluded)

Day	N in Urine	N in Hair-Feces and Cage Wash	Total N Output	N in Food	N Balance	
Second Fast						
1	1.081	0.054	1.135		-1.135	
2	1.197	"	1.251		-1.251	
3	1.209	"	1.263		-1.263	
4	1.202	"	1.256		-1.256	
5	1.251	"	1.305		-1.305	
6	1.471	"	1.525		-1.525	
7	1.128	"	1.182		-1.182	
8	1.368	"	1.422		-1.422	
9	1.218	"	1.272		-1.272	
10	1.194	"	1.248		-1.248	
11	1.158	"	1.212		-1.212	
12	1.332	"	1.386		-1.386	
13	1.357	"	1.411		-1.411	
14	1.182	"	1.236		-1.236	
15	1.207	"	1.261		-1.261	
16	1.344	"	1.398		-1.398	
17	1.119	"	1.273		-1.273	
18	1.193	"	1.247		-1.247	
19	1.335	"	1.389		-1.389	
20	1.257	"	1.311		-1.311	
21	1.221	"	1.275		-1.275	
22	1.203	"	1.257		-1.257	
23	1.315	"	1.369		-1.369	
24	1.216	"	1.270		-1.270	
25	1.145	"	1.199		-1.199	
26	1.126	"	1.180		-1.180	
27	1.495	"	1.549		-1.549	
28	1.519	"	1.573		-1.573	
29	2.089	"	2.143		-2.143	
30	2.298	"	2.352		-2.352	

TABLE V
Body Weight and Creatinine Coefficient

Day	Body Weight Kgs.	Gross Gain or Loss Kgs.	Daily Gain or Loss Kgs.	Per cent Gain or Gross Loss	Per cent Gain or Daily Loss	Creatinine Coefficient
Preliminary Period						
6	3.42					0.013
7	3.40					0.012
8	3.41					0.012
First Fast						
1	3.26	-0.15	-0.15	4.40	4.40	0.009
2	3.19	-0.22	-0.07	6.45	2.15	0.009
3	3.13	-0.28	-0.06	8.21	1.88	0.009
4	2.95	-0.46	-0.18	13.49	5.75	0.010
5						0.012
6	2.91	-0.50	-0.04	14.66	1.35	0.008
7	2.86	-0.55	-0.05	16.13	1.72	0.010
8	2.77	-0.64	-0.09	18.77	3.15	0.009
9						0.009
10	2.60	-0.81	-0.17	23.75	6.14	0.010
11	2.49	-0.92	-0.11	26.98	4.23	0.009
12	2.31	-1.10	-0.18	32.26	7.23	0.008
13	2.22	-1.19	-0.09	34.60	3.90	0.006
14	2.05	-1.36	-0.17	39.88	7.66	0.005
15	1.85	-1.56	-0.20	45.75	-9.75	0.008
Preliminary Feeding						
1	1.95	+0.10	+0.10	6.41	+5.40	0.005
2	2.25	+0.40	+0.30	25.63	+15.38	0.006
3	2.35	+0.50	+0.10	32.04	+4.44	0.006
4	2.24	+0.39	-0.11	24.99	-4.68	0.006
Normal Diet						
5	2.20	+0.35	-0.04	22.43	-1.78	0.006
6	2.12	+0.27	-0.08	17.30	-3.64	0.008
7	2.19	+0.34	+0.07	21.80	+3.30	0.008
8	2.24	+0.39	+0.05	24.99	+2.28	0.008
9	2.25	+0.40	+0.01	25.63	0.45	0.008
10	2.26	+0.41	+0.01	26.27	0.44	0.009
11	2.27	+0.42	+0.01	26.91	0.44	0.009
12	2.29	+0.44	+0.02	28.20	0.88	0.008

TABLE V (con)

Day	Body Weight	Gross Gain or Loss	Daily Gain or Loss	% Gain or Gross Loss	% Gain or Daily Loss	Creatinine Coefficient
Normal Diet (con)						
13	2.28	+0.43	-0.01	27.56	-0.44	0.008
14	2.31	+0.46	+0.03	29.48	+1.31	0.008
15	2.34	+0.49	+0.03	31.40	1.29	0.010
16	2.36	+0.51	+0.02	32.68	0.85	0.010
17	2.36	+0.51	0.00	32.68	0.00	0.009
18	2.36	+0.51	0.00	32.68	0.00	0.009
19	2.38	+0.53	+0.02	33.96	0.85	0.011
50% Increase in Diet						
20	2.42	+0.57	+0.04	36.53	1.68	0.011
21	2.49	+0.64	+0.07	41.01	2.89	0.011
22	2.50	+0.65	+0.01	41.65	4.01	0.010
23	2.57	+0.72	+0.07	46.14	2.80	0.011
24	2.61	+0.76	+0.04	48.70	1.55	0.011
25	2.63	+0.78	+0.02	49.98	0.77	0.012
26	2.67	+0.82	+0.04	52.55	1.52	0.011
27	2.75	+0.90	+0.08	57.67	3.00	0.013
100% Increase in Diet						
28	2.84	+0.99	+0.09	63.44	3.27	0.013
29	2.85	+1.00	+0.01	64.08	0.35	0.012
30	2.92	+1.07	+0.07	68.57	2.45	0.012
31	3.00	+1.15	+0.08	73.70	2.74	0.012
32	3.00	+1.15	0.00	73.70	0.00	0.012
33	3.09	+1.24	+0.09	79.46	3.00	0.013
34	3.10	+1.25	+0.01	80.10	0.32	0.012
35	3.20	+1.35	+0.10	86.50	3.22	0.012
36	3.24	+1.39	+0.04	89.08	1.25	0.012
37	3.32	+1.47	+0.08	94.20	2.47	0.013
38	3.35	+1.50	+0.03	96.12	0.90	0.013
39	3.40	+1.55	+0.05	99.33	1.49	0.013
40	3.46	+1.61	+0.06	103.17	1.76	0.012
41	3.50	+1.65	+0.04	105.74	1.16	0.013
42	3.52	+1.67	+0.02	107.02	0.57	0.013
43	3.56	+1.71	+0.04	109.58	1.14	0.013
44	3.59	+1.74	+0.03	111.50	0.84	0.012

TABLE V (concluded)

Day	Body Weight	Gross Gain or Loss	Daily Gain or Loss	% Gain or Gross Loss	% Gain or Daily Loss	Creatinine Coefficient
Normal Diet						
45	3.56	+1.71	-0.03	109.58	-0.83	0.011
46	3.54	+1.69	-0.02	108.30	-0.56	0.012
47	3.54	+1.69	0.00	108.30	0.00	0.011
Second Fast						
1	3.42	-0.12	-0.12	3.39	-3.39	0.009
2	3.32	-0.22	-0.10	6.21	2.92	0.009
3	3.26	-0.28	-0.06	7.91	1.81	0.009
4	3.11	-0.43	-0.15	12.15	4.60	0.010
5	3.07	-0.47	-0.04	13.28	1.28	0.010
6						
7	3.05	-0.49	-0.02	13.84	0.651	0.009
8	2.95	-0.59	-0.10	16.66	3.28	0.009
9	2.89	-0.65	-0.06	18.36	2.03	0.008
10	2.86	-0.68	-0.03	19.21	1.04	0.009
11	2.80	-0.74	-0.06	20.90	2.10	0.008
12	2.77	-0.77	-0.03	21.75	1.07	0.010
13	2.72	-0.82	-0.05	23.16	1.80	0.009
14	2.68	-0.86	-0.04	24.29	1.47	0.009
15	2.64	-0.90	-0.04	25.42	1.49	0.009
16	2.58	-0.96	-0.06	27.12	2.27	0.008
17	2.51	-1.03	-0.07	29.09	2.71	0.006
18	2.49	-1.05	-0.02	29.66	0.79	0.008
19	2.45	-1.09	-0.04	30.79	1.60	0.008
20	2.40	-1.14	-0.05	32.20	2.04	0.008
21	2.37	-0.03	-0.03	33.05	1.25	0.007
22	2.36	-1.18	-0.01	33.33	0.42	0.006
23	2.31	-1.23	-0.05	34.74	2.12	0.006
24	2.21	-1.33	-0.10	37.57	4.33	0.005
25	2.18	-1.36	-0.03	38.42	1.35	0.005
26	2.15	-1.39	-0.03	39.26	1.37	0.006
27	2.11	-1.43	-0.04	40.39	1.86	0.007
28	2.05	-1.49	-0.06	42.09	2.84	
29	1.97	-1.57	-0.08	44.35	3.90	
30	1.91	-1.63	-0.06	46.04		

TABLE VI
Nitrogen in Diet - Feces - Hair and Cage Washings.

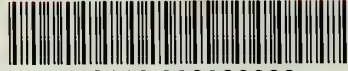
	Preliminary Period 11-days	First Fast. 15-days	Feeding Period					Second Fast.
			Preliminary 4-days	Normal 15-days	50% Increase 8-days	100% Increase 17-days	Normal 3-days	
Food (per day)	Meat	2.533	^x 3.618	2.533	3.799	6.666	2.533	
	Crackers	0.1977	^x 2.930	0.1977	0.303	0.395	0.1977	
	Lard	0.0008	^x 0.003	0.0008	0.0012	0.002	0.0008	
	Bone ash	0.0003	^x 0.003	0.0003	0.0005	0.0005	0.0003	
	Water a.c.	250 cc.	250 cc	250 cc	250 cc	250 cc	250 cc	250 cc
	Total	2.732	^x 6.554	2.732	4.104	5.464	2.732	
Feces	Grams	41.89	3.5	76.6	61.0	92.0	20.0	9.6
	Wt of N	1.691	0.309	2.143	1.596	3.540	0.739	0.776
	% N	4.037	8.874	2.798	2.616	3.839	3.695	8.106
	Grs %/day	0.154	0.021	0.113	0.199	0.208	0.269	0.026
Hair	Grams	5.0		13.0				6.0
	Wt of N	0.575		1.555				0.666
	% N	11.50		11.961				11.100
	Grs %/day	0.052		0.025				0.022
Cage Washings	Wt of N	0.326	0.268	1.084 (47 days)				0.189
	Grs %/day	0.029	0.0178	0.023				0.006
Grams N Excreted		2.592						16.31
	Grams N per day.	0.235	0.064	0.161	0.247	0.256	0.317	0.054

^x Total weight of nitrogen fed during the four days





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